Characterization and mapping of dipolar interactions within macromolecules in tissues using a combination of DQF, MT and UTE MRI

Uzi Eliav\(a\), Michal Komlosh\(b,c\), Peter J. Basser\(b\) and Gil Navon\(a\)*

This study shows that by combining a double-quantum filtered magnetization transfer (DQF-MT) with an ultra-short TE (UTE) MRI that it is possible to obtain contrast between tissue compartments based on the following characteristics: (a) the residual dipolar coupling interaction within the biomacromolecules, which depends on their structure, (b) residual dipolar interactions within water molecules, and (c) the magnetization exchange rate between biomacromolecules and water. The technique is demonstrated in rat-tail specimens, where the collagenous tissue such as tendons and the annulus pulposus of the disc are highlighted in these images, and their macromolecular properties along with those of bones and muscles can be characterized. DQF-MT UTE MRI also holds promise because collagenous tissues that are typically invisible in conventional MRI experiments produce significant signal intensities using this approach. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: DQF; MT; UTE; dipolar interaction; rat tail; tendons; annulus pulposus; intervertebral disc; short T2

INTRODUCTION

Commonly used clinical MRI methods use water magnetization to obtain anatomical and functional information based on diffusion coefficients and relaxation times. However, these methods provide only limited information about the properties of biomacromolecules (MM) such as collagen that are common in extracellular matrix (ECM) and connective tissues such as cartilage, ligaments and tendons. To obtain better insight into the properties of MM content of the tissue, it is desirable to have methods that probe MM magnetization. Attempts to achieve this goal have used magnetization transfer contrast (MTC). In that method, MM magnetization is fully saturated and the decline in the water signal is monitored. Results depend on the longitudinal relaxation (\(T_1\)) of water and its rate of exchange with the MM, and thus provide only limited information about the identity and nature of MM. It has been argued, however, that by considering the level of \(B_1\) and the frequency offset from the water, that MTC MR might yield a distribution of \(T_2\) values of the MM (1).

Another method to select the subpopulation of MM-associated protons is with double quantum filtering (DQF) MRI (2–6). This approach allows for the selection of the protons to be excited based on the strength of their dipolar interactions and is therefore more sensitive to MM properties, whereby facilitating contrast based on these properties. Since water molecules are highly mobile their intramolecular dipolar interactions largely average out. This characteristic can be used to suppress the water signal and retain only the MM protons whose motions are highly restricted and whose dipolar interactions do not entirely average out. Indeed, spectroscopic studies of MM such as collagen yielded a spectral width of tens of kHz. Such a large spectral width cannot be detected by MRI, even with methods that use a very short echo time (TE). In previous studies of neuronal systems, this difficulty was solved by using DQF-MT, where the MM transverse magnetization was converted to the longitudinal axis and subsequently to water magnetization by proton exchange (3) whose magnetization could be used for imaging (6). Though this method provides detailed information about MMs in neuronal systems, its application to connective tissues is still restricted by the short water transverse relaxation time (\(T_2\)), thereby limiting the use of popular imaging methods such as gradient and spin-echo MRI and requiring large slice thicknesses and low voxel resolutions. These limitations have made the study of MM content and composition difficult. Recently, methods using ultra-short TE (UTE) have enabled imaging of tissues with short \(T_2\) such as tendons. (7–10). As a result, in the present study, DQF-weighted NMR was combined with UTE MRI to obtain images with contrasts that distinguished tissue compartments based on differences in their MM content.

* Correspondence to: G. Navon, School of Chemistry, Tel Aviv University, Levanon Street, Tel Aviv, 69978, Israel. E-mail: navon@post.tau.ac.il

a U. Eliav, G. Navon
School of Chemistry, Tel Aviv University, Tel Aviv, Israel

b M. Komlosh, P. J. Basser
Section on Tissue Biophysics and Biomimetics (STBB), Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), NIH, Bethesda, MD, USA

c M. Komlosh
Center for Neuroregenerative Medicine (CNRM) and the Henry Jackson Foundation (HJF), Bethesda, MD, USA

Abbreviations used: DQF, double-quantum filter; ECM, extracellular matrix; GE, gradient echo; MM, macromolecules; MT, magnetization transfer; MTC, magnetization transfer contrast; TE, time to echo or echo-time; UTE, ultra-short TE; FID, free induction decay.
Dipolar interaction between nuclei is a function of their distance (1/r^3) and orientation of the internuclear vector with respect to the external magnetic field. In well-ordered systems, this interaction leads to a well-resolved spectrum. In systems where the orientations of the internuclear vectors are partially ordered or randomly distributed, the spectral lineshape will vary between a Pake doublet and a Gaussian, respectively. Moreover, dynamic processes such as reorientation and chemical exchange cause reductions in the observed dipolar interaction to an extent that depends on the rate and anisotropy of the motion. For MMs such as collagen in connective tissues for which the motions are slow or anisotropic and where chemical exchange has no effect, dipolar interactions between protons are not significantly reduced (having order parameters in the range 0.3-1.0) and their size is in the tens of kHz. Such a large interaction manifests itself with spin dynamics on a time scale of a few tens of μs. For bound water molecules on the other hand, the intramolecular dipolar interactions are significantly reduced as a result of their fast isotropic or anisotropic reorientational motion, their exchange with the bulk water, and the exchange between binding sites with different average orientations. Furthermore, proton exchange between water molecules causes an additional reduction in the proton-proton intramolecular dipolar interaction (3).

The latter process is affected by temperature (3), pH (Eliav, Seo and Navon, unpublished results), and buffer concentration (11).

It has been shown that 1H water NMR spectra in ordered systems such as collagen fibres and tendons, where proton exchange among water molecules were suppressed either by cooling or by reducing the amount of free water, resulted in a splitting due to residual dipolar interactions (3,12,13). For water, typical residual dipolar interactions were less than 1 kHz and therefore, the spin dynamics occurred on a time scale of hundreds of μs. When the rate of proton exchange between water molecules was greater than the residual dipolar interaction, coalescence of splitting occurred and the linewidth was given by 1/T2 = ω0^2/k, where ω0 is the dipolar interaction and k is the proton exchange rate (3).

Dipolar interaction leads to the formation of high rank tensors such as l1l2l3 (1 and 2 indicate the interacting protons) that can be selected by DQF (14). It has further been shown that the intensity of this observed signal might depend on the dipolar interaction ωD, making it possible to distinguish among compartments by these interactions (3,6). However, the population of immobile or restricted MM-associated protons that can be selected and excited using DQF are not sufficient in themselves to produce a strong MR signal suitable for MRI acquisition due to their large ωD, which results in a fast decay of the MM transverse magnetization. As a result, another step is required to amplify the signal due to the MM-associated protons. This is performed using an MT approach presented in the DQF-MT pulse sequence described below (Fig. 1). This method enables to study the effects of the residual dipolar interaction in tissues, to gain information about water intramolecular dipolar interaction and MM content, and to obtain a new and measurable contrast by MRI on the basis of this information. In Figure 1, gradient echo (GE) is used as an imaging block. However, in the following, we also show results where 2D ultra short TE (2D-UTE) is used for image formation. For spectroscopic studies, the imaging module was replaced by a single 90° pulse followed by signal acquisition.

**Figure 1.** Schematic presentation of the DQF-MT MRI pulse sequence using the gradient-echo imaging modality. The duration of 90° hard pulses was 22 μs. The phase cycling was eight steps given by: q1 = q2 = 0° 90° 180° 270°, q4 = q3 = 0° 90° 90° 90°; q5 = 0°; q6 = 0° 180° 180° 180° 0°. Isotropic anisotropic exchange bound spin-1/2 protons were excited by the 180° pulse. Temporal evolution of the quantum coherences T1, T2, T1, T2 is given in the table.
and water occurred. In the following discussion, we redefine the time of pseudo-equilibrium as $t_{\text{LM}} = 0$. For the case where at pseudo-equilibrium only MM magnetization was retained, the following conditions applied: $m_{\text{zw}(t_{\text{LM}} = 0)} = 0$ and $m_{\text{zp}}(t_{\text{LM}} = 0) 
eq 0$.

In the following discussion, $m_{\text{zp}}$ and $m_{\text{zp}}$ are the longitudinal magnetizations of water and MM's, respectively, that were obtained after the DQ filter immediately after pseudo-equilibrium was established. As a result, only the transfer of the magnetization $m_{\text{zp}}$ ($t_{\text{LM}} = 0$) to water during the $t_{\text{LM}}$ period was observed in the experiment shown in Figure 1. In previous studies of tendons (4,6), it was shown that the transfer mechanism between MM and water is dominated by proton exchange between them. In addition, since the two most visible compartments in the images of rat tail described below consisted of collagen, it can be assumed that proton exchange is the most important transfer process in the rat tail. From a mathematical prospective while conducting phase cycling, the effect of the phases of the RF pulses that preceded the time interval $t_{\text{LM}}$ was to multiply $m_{\text{zp}}(t_{\text{LM}} = 0)$ by the phase $e^{i\phi_{p}}$, where $\phi_{p} = \pm (\phi_{1} + \phi_{2})$ (Eq. [2a]) and by $e^{-i\phi_{w}}$ during acquisition (Eq. [2b]). For a single site, the detected MM magnetization $m_{p}$ ($m_{p} = m_{p}^{(\text{eq})} + m_{p}^{(\text{aw})}$) obtained after the last pulse is given by Equation [2a]:

$$m_{p} = \frac{\sqrt{2}}{2} \left( m_{\text{zp}}(t_{\text{LM}} = 0) e^{-\frac{\omega}{T_{1p}} + m_{\text{eq}}^{(\text{aw})} \left( 1 - e^{-\frac{\omega}{T_{1p}}} \right)} \right)$$  \[2a\]

$$m_{p} = \frac{\sqrt{2}}{2} \left( m_{\text{zp}}(t_{\text{LM}} = 0) e^{-\frac{\omega}{T_{1p}} + e^{-i\phi_{w}} m_{\text{eq}}^{(\text{aw})} \left( 1 - e^{-\frac{\omega}{T_{1p}}} \right)} \right)$$  \[2b\]

In this expression, there are two contributions to $m_{p}$: the first originating from the magnetization immediately after the fourth 90° pulse that is multiplied by the phase $e^{i\phi_{p}}$ and the second from the magnetization formed during the interval $t_{\text{LM}}$ by longitudinal relaxation, which is not multiplied by that phase. During acquisition following the last pulse, both contributions to the signal were multiplied by the phase $e^{-i\phi_{w}}$ (Eq. [2b]). As a result of the phase cycling on $\phi_{w}$, the second term in Equation [2b], which depends on the longitudinal relaxation and not on the transverse magnetization spin dynamics within the MM, was eliminated (Eq. [2c]).

For exchange between the two sites, it was shown in a previous study (4) that under the initial conditions mentioned above ($m_{\text{zw}}(t_{\text{LM}} = 0) = 0$, $m_{\text{zp}}(t_{\text{LM}} = 0) \neq 0$) and the fast exchange limit ($k > > 1/T_{1}$), where $k$ is the sum of the MT rates from the MM to the water and that of the reverse reaction, the detected magnetizations of water and MM $m_{w}$ and $m_{p}$, respectively, are given in Equation [3]:

$$m_{p} = \frac{\sqrt{2}}{2} m_{\text{zp}}(t_{\text{LM}} = 0) \left( p_{w} e^{\frac{-t_{\text{LM}}}{T_{1p}}} + p_{p} e^{\frac{-t_{\text{LM}}}{T_{1p}}} \right)$$  \[3\]

$$m_{w} = \frac{\sqrt{2}}{2} m_{\text{zp}}(t_{\text{LM}} = 0) p_{w} \left( e^{\frac{-t_{\text{LM}}}{T_{1p}}} - e^{\frac{-t_{\text{LM}}}{T_{1}} - e^{\frac{-t_{\text{LM}}}{T_{1}}}} \right)$$

$$\frac{1}{T_{1}} = p_{w} R_{1w} + p_{w} R_{1w}$$

where $m_{\text{zw}}(t_{\text{LM}} = 0) = m_{\text{zw}}^{(\text{eq})} \sin^{2} \left( \omega_{0} T_{2} + 2 \right) e^{-i/T_{2}}$, $m_{\text{zp}}^{(\text{eq})}$, $\omega_{0}$, and $T_{2}$ are the MM equilibrium magnetization, intramolecular dipolar interaction and transverse relaxation, respectively, and $R_{1w} = l/T_{1w}$.

The longitudinal relaxation times of MMs and water, respectively. The fractions of the protons residing on the MMs and in water are given by $p_{w}$ and $p_{p}$, respectively. From Equation [3], it is clear that during MT the water signal is given by a difference of two exponentials: one that characterizes the buildup due to an exchange process and the other that describes the decay due to longitudinal relaxation (with rate given by $1/T_{1}$). Experimentally, this was confirmed in previous studies of MMs and water signals (4,6) and in the current study for MMs (Figs. 3, 4) where it is shown that for long $t_{\text{LM}}$, the signal decayed off. More generally, longitudinal relaxation was observed as a decay process when prior to its monitoring phase, cycling that selected passage through the transverse plane was implemented.

In addition to the changes in the water signal, MM magnetization was decaying bi-exponentially. It is worth noting that for $t_{\text{LM}}/T_{1} << 1$, Equation [3] shows that the sum of the water and MM magnetizations equal $m_{\text{zp}}(t_{\text{LM}} = 0)$. However, for widely used conditions in studies of tissues, $t_{\text{LM}} > 1/k$, $t_{\text{LM}} << T_{1}$ and $p_{w} \approx 1$, the water signal, is proportional to $m_{\text{zp}}(t_{\text{LM}} = 0)$. This result is summarized in Equation [4]:

$$m_{\text{zw}}(t_{\text{LM}} \text{max intensity}) = m_{\text{zp}}(t_{\text{LM}} = 0)$$  \[4\]

It is worth noting that under the conditions that make Equation [4] valid, the MT between the MMs and the water had reached chemical equilibrium but the effect of longitudinal relaxation was negligible. For the case of selective excitation of the water, expressions similar to those in Equation [3] were obtained by interchanging the subscripts $p$ and $w$.

From the above discussion, it follows that the DQF-MT sequence enables the experimenter by judicious choice of $\tau$ and $t_{\text{LM}}$ to obtain MRI contrast that reflects differences among various compartments based on: (a) the number of the MM protons, (b) MM dipolar interactions, and (c) exchange rates between MMs and water. In the following sections, we demonstrate for several systems the effect of the tissue properties such as order, MM content, and exchange rate on the images obtained with the pulse sequences DQF-MT (Fig. 1).

### EXPERIMENTAL

Spectroscopic NMR and MRI experiments were conducted on a 14.1 T Bruker micro-imager (Bruker Biospin, Germany). For imaging studies, the pulse sequence shown in Figure 1 was used either with GE as shown or with a commercially available 2D ultra-short TE pulse sequence (Bruker’s 2DUTE program running within Bruker’s operating system Paravision version 5.1) (7–10): For spectroscopic purposes, the MRI module was replaced by a single 90° pulse. The hard 90° pulses shown in Figure 1 were 22 $\mu$s. The ranges of values examined for $\tau$ and $t_{\text{LM}}$ were 5–400 $\mu$s and 0.001–600 ms, respectively. The UTE method was optimized by comparison with gradient echo (GE) images of a porcine spinal cord that had a $T_{2}$ relaxation time longer than the TE used in the GE method (3.1 ms). The combined DQF-MT and UTE experiments were performed on the part of the rat tail that is the closest to the animal’s body. It was skinned, immersed in perfluoropolyether (Fomblin® LC/8, Solvay Solexis, Italy), and then placed in a 10-mm Shigemi tube (Shigemi, Japan) to reduce susceptibility artefacts. The samples were aligned with their cylindrical symmetry axis parallel to the magnetic field.
RESULTS AND DISCUSSION

NMR Spectroscopy

In Figure 2, spectra of rat tail obtained by DQF-MT are shown as a function of the creation/reconversion periods; \( t/2 \) at very short (10 \( \mu \)s) \( t_{LM} \). Two types of spectra are evident. For \( t \leq 70 \mu \)s, a very broad component with a line width > 10 kHz was observed (Fig. 2a), while for longer time scales, a single peak of \( \sim 900 \) Hz line width emerged (Fig. 2b), reaching a maximum intensity for \( t/2 \) in the range of 300-400 \( \mu \)s. The width of this peak was maintained over a large range of creation/reconversion times (25-600 \( \mu \)s) and thus can be attributed to a single species.

By setting \( t/2 \) in the range of 5-80 \( \mu \)s (typical for MM transverse magnetization, 4,6) and examining the dependence on \( t_{LM} \), we obtained the spectra shown in Figure 3. Two major physical processes are noted in Figure 3 (See also ref. 4). On a time scale, shorter than 0.8 ms fast-spin diffusion within the MMs caused the spectrum to converge to an unchanging shape for \( t_{LM} > 0.8 \) ms (Fig. 3a) but its amplitude decayed off with a concomitant increase in a narrow peak that was attributed to water. This assignment was based on previous spectroscopic studies on tendon, where a similar peak of \( \sim 900 \) Hz width was attributed to the water, since it disappeared upon replacement of the H\(_2\)O in the sample with D\(_2\)O. This peak reached its maximum intensity at \( t_{LM} = 100 \) ms. The intensity of the MMs decayed by a factor of \( \sim 4 \) for \( t_{LM} = 12 \) ms and by at least 8 for \( t_{LM} = 100 \) ms. Since no narrow peak was observed at short \( t_{LM} \), it is clear that the water peak seen at \( t_{LM} = 100 \) ms originated solely from magnetization transfer from the MMs. It is evident from Figure 3b that during the exchange process, two peaks of different line widths (both < 1 kHz) were formed. One of the peaks had a width of 900 Hz while the other had a line width of 70 Hz (T\(_2\) ~ 4.5 ms). The 900 Hz line resembled the water signal in tendon (T\(_2\) ~ 350 \( \mu \)s). An estimate of 1~1.5 s for T\(_1\) of the above two components was obtained from the decay of the signals as shown in Figures 3b and 4b. The narrower of the two peaks might be identified with the water signal in the annulus fibrosus, since its T\(_2\) was reported to be 19 ms (15), which is compatible with the width reported in the current study, taking into account possible inhomogeneity and susceptibility effects. Contribution to this peak from the nucleus pulposus was expected to be limited since it contained small amounts of collagen, while proteoglycans represent the major fraction of the nucleus’ MM content. These proteoglycans did not contribute to the DQF-MT since their high mobility reduced their residual dipolar interaction. This assignment is further supported by the imaging results described in the next section.

![Figure 2](image1.png)

**Figure 2.** Dependence of \( ^1H \) rat tail spectrum on creation/reconversion times \( t/2 \) using the pulse sequence shown in Figure 1 (DQF-MT) where the imaging modality was replaced by a single 90\(^\circ\) pulse. The shown spectra are drawn on two spectral ranges: (a) the tens of kHz range suitable to display the MM spectra; (b) the few kHz range suitable to display the water molecules spectra. \( t_{LM} = 1.5 \mu \)s.

![Figure 3](image2.png)

**Figure 3.** Dependence of \( ^1H \) rat tail spectrum on exchange time \( t_{LM} \) using the pulse sequence shown in Figure 1 (DQF-MT) where the imaging modality was replaced by a single 90\(^\circ\) pulse. The shown spectra are drawn on two spectral ranges: (a) the tens of kHz range suitable to display the MM spectra; (b) the few kHz range suitable to display the water molecules spectra. The creation/reconversion time \( t/2 \) was 10 \( \mu \)s.
In Figure 4, images of an axial slice through a disc of the most superior portion of the rat tail were obtained by gradient echo (GE) and UTE and the latter method weighted by DQF-MT. In the GE images with TE of 2.86 ms, the tendons are absent. Only the annulus fibrosus and the nucleus pulposus are visible as well as the thin layers of para-, epi- and peri-tendineum (16) surrounding the tendons. The UTE method makes the tendons appear with positive contrast, reflecting the fact that weighting by T2 is less important than in GE. Since the duration of the gradients used for the imaging were 2 ms (both for GE and UTE), which is too long to image macromolecules such as collagen that dephase on a time scale shorter than 80 μs, the contrast obtained by UTE was mostly due to the proton density of the water molecules. However, the short TE diminished the contrast between the annulus fibrosus and nucleus pulposus as well as between the tendons and their surrounding tissue, i.e. the SNR of the tendons, annulus fibrosus and muscles were similar (Fig. 4). This shortcoming was addressed by combining UTE with DQF-MT (bottom of Fig. 4) with the creation/reconversion times set to 300 μs. In this image, only the tendons are visible, thus confirming that the peak of 900 Hz width (Fig. 1) was due to residual dipolar interactions of bound water in tendons. It is worth noting that the para-tendineum (16) in close proximity to the tendons was absent from the DQF-MT-weighted UTE MRI, although present in the UTE images. As explained in the theoretical background section, combining DQF-MT and UTE can yield images with contrasts that reflect differences in MM characteristics. For rat tail, it was expected to distinguish between various types of collagen that constitute the tendons and the annulus fibrosus, e.g. Type I and Type II collagens. In Figure 5, we show UTE MRIs weighted by a DQF-MT sequence. As can be observed, only two parts of the rat tail are clearly visible: the tendons and the annulus, while there is some contribution from muscles in the images obtained for τ/2 = 10 μs. For this value of τ/2 and t\textsubscript{LM} in the range of 50–600 ms, the ratio of the SNR of tendon and annulus fibrosus was 1.6, i.e. very different from the equal value observed in the UTE images. Furthermore, since spectroscopic measurements showed that for τ/2 = 10 μs, the contribution of intramolecular dipolar interaction of the water was negligible and the contrast in the UTE images very small, we attribute the contrast between the tendon and annulus to differences in collagen type or content. This conclusion is consistent with the known difference in the collagen composition of tendons and annulus (17). Tendons mostly consist of collagen type I, while
the annulus contains both types I and II collagen (18) with the former being abundant in the outer part of the annulus and the latter in the inner part closer to the nucleus pulposus. Examining the dependence of the images on the creation/reconversion periods t/2, we observed that the signals from the tendons, annulus and muscles decayed off on the time scale of tens of μs. However, the extent of their intensity fall-off resulting from increasing t/2 from 10 to 40 μs was different, namely by factors of 1.0, 2.0, and 1.5 for tendons, annulus fibrosus, and muscles, respectively. Therefore, we concluded that on a creation/reconversion time scale of tens of μs, it was the MM spin dynamics that dominated the MRI signal. This conclusion is further supported by the fast decay of the DQ coherence during the time interval tDQ occurring on a time scale shorter than tDQ = 80 μs. This fast decay can be the sole result of spin systems where two dipolar interactions are involved: for instance, dipolar interaction between protons in one CH2 group and protons in another CH2 group or in CH or CH3 groups. It is worth noting that the lack of decay of the tendon’s signal could be the result of a combined effect of a decay of the MM magnetization compensated by a signal buildup due to the intramolecular dipolar interaction of the water. This buildup could be more significant for the tendon relative to other tissues in the rat tail based on the larger residual interaction of water in the tendon. From spectroscopic studies, the contribution from the water residual intramolecular interaction at t/2 = 40 μs can be as large as 40%, as was estimated in the following manner: upon changing t/2 from 10 μs to 40 μs with tLM = 1 μs, the water signal increased by a factor of 3.5 while MM intensity decreased by a factor of 3. Since the amount of magnetization that can be transferred to water is proportional to MM magnetization, a simple calculation yields that at least 40% of the water signal in tendon acquired with t/2 = 40 μs and 100 ms < tLM < 300 ms can be attributed to a buildup due to water residual intramolecular dipolar interaction. Since the water residual intramolecular interaction in the annulus and muscle was small, the water DQ-MT signal buildup at t/2 = 40 μs due to this interaction can be neglected. As a result, the difference in the decay of the signals as a function of t/2 between these two tissues was solely due to differences in their MM structures.

For short creation times, the dependence on the exchange period tLM showed a maximum at 200 ms and was similar for tendons and the annulus. This similarity might suggest that the exchange with water proceeded through similar functional groups present on the collagen backbone, i.e. labile protons of hydroxyproline or hydroxylysine. Further demonstration of the capability of DQ-MT when combined with UTE to distinguish between tissues based on their MM differences is shown in Figure 6, where an axial slice through a vertebrae located in the middle between two discs is shown. As in the case

**Figure 5.** Images of axial slice of rat tail through disc that was obtained using DQF-MT combined with UTE are shown as a function of creation/reconversion time t/2 and exchange time tLM. Matrix size = 256 x 256, slice thickness = 2.1 mm. The selective RF pulse duration was 0.1 ms. Dead time after echo slice was 2 μs, TR = 2 s, number of averages = 8. The SNR of the tendons (t) was calculated as an average of the SNRs of all tendons and similarly, the SNR for annulus (a) was calculated by taking into account all the area of this tissue.
of the disc, the contrast between the various tissues in the DQF-MT-weighted UTE images were very different from the one obtained by UTE. For the UTE experiment, SNR’s of 6.8, 8.8 and 7.2 were obtained for the tendon, muscle and bone, respectively (Fig. 6). The addition of DQF-MT weighting with \( \tau/2 = 10\,\mu\text{s} \) eliminated the contribution from the bone marrow and set the SNRs (for eight scans) of the tendons, muscles and vertebrae to 8.7, 4.6 and 2.9, respectively. Using the same arguments as in the discussion of the results obtained with \( \tau/2 = 10\,\mu\text{s} \) for the disc, we might conclude that differences between the various tissues reflected differences in MM contents and structure. Furthermore, examining the dependence of the images on the creation/reconversion periods, \( \tau/2 \) showed that tendons, muscles and vertebrae decayed on a time scale of tens of \( \mu\text{s} \), indicating that the decay was dominated by MM magnetization. However, the extent of the intensity decay as a result of increasing \( \tau/2 \) from 10 to 40 \( \mu\text{s} \) was different: 1.3, 1.9 and 1.5 for tendons, muscles and vertebrae respectively, reflecting differences in the MM structure.

**CONCLUSIONS**

In the present work, we demonstrated that combining a DQF-MT “filter” with UTE MRI produced image contrast that was based on the residual dipolar interactions, either within the water molecules or the macromolecules. It was further demonstrated that this new type of contrast makes it possible to distinguish among various compartments within the rat tail on the basis of differences in their macromolecular properties. The prospect of possibly being able to distinguish among different types of collagen could have far-reaching consequences in diagnostic applications, particularly in orthopaedics where there is a dearth of successful MR methods for imaging ECM, intervertebral discs, cartilage, bone, tendons and ligaments.

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